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ISOLATION OF LYTIC AGENTS RELATED TO SPHAEROTILUS

by

Dorothy J. Geason
Randolph-Macon Woman's College
Lynchburg, Virginia 24503

for the

FEDERAL WATER POLLUTION CONTROL ADMINISTRATION

DEPARTMENT OF THE INTERIOR

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27 Abstract

This study has been undertaken to isolate and identify those agents found in nature which are capable of lysing Sphaerotilus and thus abate the nuisance associated with its occurance in flowing streams and raw sewage. Enrichment culture of river water or raw sewage and either a locally isolated strain or ATCC 15338 has resulted in the isolation of four bacteria capable of destroying the cells but not the sheath of Sphaerotilus. Specific bacteriophage could not be demonstrated. Bdellovibrio bacteriovorus first appeared as small motile rods causing spheroplasting and lysis of the host. After subsequent passages through the host it increased in numbers, size, and avidity of attack. Two other isolates were gram-negative rods which formed microcysts but not fruiting bodies, and produced non-diffusible yellow or red pigments. These have been tentatively identified as myxobacters of the genus Sporocytophaga. The fourth isolate is also a gram-negative rod. It forms extremely mucoid colonies and is being further studied. (EPA abstract)

Abstractor Dorothy J. Geason	Institution Randolph-Macon Woman's College

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ABSTRACT

The proliferation of filamentous bacteria of the genus Sphaerotilus in flowing streams and raw sewage has been cited as the cause of a number of undesirable effects. This study has been undertaken to isolate and identify those agents found in nature which are capable of lysing Sphaerotilus and thus serve to abate the nuisance factor associated with this organism.

Enrichment culture of river water or raw sewage and either a locally isolated strain or ATCC 13338 has resulted in the isolation of four microorganisms capable of destroying the cells of Sphaerotilus. Specific bacteriophage could not be demonstrated.

The isolate considered to be the ectoparasitic bacterium Bdellovibrio bacteriovorus first appeared as a very small motile vibrio causing spheroplasting and lysis of Sphaerotilus. After subsequent passages through the host it increased in numbers, size and avidity of attack.

Two isolates are tentatively identified as myxobacters of the genus Sporocytophaga. These are both gram-negative rods which form microcysts but not fruiting bodies. A fourth isolate, also a gram-negative rod is being further studied for identification.

The object of this research has been to elucidate basic principles regarding the ecology of Sphaerotilus which might be useful to sanitary engineers and concerned idustries in their management of waste products in regard to pollution control.

This report was submitted in fulfillment of Grant No. 17050 DHI between the Federal Water Pollution Control Administration and Dorothy J. Geason, Research Associate, Randolph-Macon Woman's College.

Key Words: Sphaerotilus, Bdellovibrio bacteriovorus, myxobacters, spheroplast formation, filamentous bacteria.

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SECTION I

CONCLUSIONS

1. The proliferation of filamentous bacteria of the genus Sphaerotilus causes a number of undesirable effects in flowing streams which receive organic wastes and in sewage treatment plants where it has been implicated in the bulking of activated sludge.

2. A strain of Bdellovibrio exists in nature which is capable of attacking and destroying the cells of Sphaerotilus.

3. At least 2 species of myxobacters that are predatory on Sphaerotilus exist in raw sewage.

4. The demonstration of the existence of bacteriophage specific for Sphaerotilus depends upon finding a method of recognizing its presence other than the clearing of broth cultures or formation of plaques on a bacterial lawn.

5. Further investigation of these microorganisms would constitute a significant contribution to basic bacteriology and to understanding the ecology of Sphaerotilus.

SECTION II

RECOMMENDATIONS

This investigation was undertaken to isolate and identify any agents capable of lysing Sphaerotilus. Those which have been found represent two very diverse groups of bacteria, each with its own growth cycle which must be understood before an evaluation can be made of their importance to the ecology of Sphaerotilus.

A continuation of the study of the Bdellovibrio isolate should place emphasis on the nature of the parasitism. The possibility that, given the proper nutrition, it can grow independent of the host should be investigated. Another question is the ultimate fate of the sheath of Sphaerotilus as the parasites escape from the spheroplasts.

The final identification of the myxobacters should be undertaken with careful attention to the nutritional requirements for the formation of microcysts and fruiting bodies.

While the scope of this study is in the area of basic bacteriology, the need for it is one which has been recognized, not only by microbiologists, but by sanitary engineers and those responsible for the management of certain industrial wastes.

SECTION III

INTRODUCTION

The proliferation of the filamentous bacteria of the genus Sphaerotilus in flowing streams, especially those which receive organic wastes, and in raw sewage causes a number of undesirable effects. It has been implicated in the fouling of nets of commercial fishers, clogging and corrosion of pipes, clogging of filters in water treatment plants and of felts in paper manufacturing machinery, and also in the bulking of activated sludge in sewage treatment plants. While these effects have long been of interest to industries and sanitary engineers, the organism itself has received little attention from microbiologists. This study was undertaken in an attempt to isolate and identify those agents which are capable of lysing Sphaerotilus.

A series of experiments was conducted in an effort to isolate a bacteriophage specific for Sphaerotilus. No phage could be detected using the conventional indicators such as the clearing of a broth culture or formation of plaques on a bacterial lawn. Due to the filamentous nature of the organism plus the fact that a phage would, in all probability destroy the cells but not the sheath, a new approach in evaluation is needed. Theoretically, the sheath, which is analogous to the microcapsule of the gram-negative enteric bacteria (10) can be removed with trichloracetic acid without the loss of viability of the cell (16). It seems clear that this or another innovative approach is needed.

The enrichment methods did, however, result in the discovery of several agents capable of destroying Sphaerotilus based upon microscopic evidence. The first of these appears to be the ectoparasitic bacterium Bdellovibrio bacteriovorus. The size of these organisms approaches the limits of resolution of the light microscope and while it increases during repeated passages through the host, the nature of the parasitism and final proof of identification must be resolved by electron microscopy. These studies are currently in progress (Appendix A).

Two other isolates have been tentatively identified according to the 7th edition of Bergey's Manual as Sporocytophaga myxococcoides and S. ellipsospora. Bicultures of each of these are being carried out at present with the aim of studying the nature of the parasitism using fluorescent antibody techniques. The fourth, an extremely mucoid isolate is in the process of identification.

SECTION IV

MATERIALS AND METHODS

Bacteria. S. natans was isolated from the James River at a point six miles below the city of Lynchburg, Virginia. It was identified according to the criteria established by Pringsheim (9) and Stokes (15). ATCC 13338, which is a strain of S. natans isolated by Stokes and used in his studies on morphology and physiology was used for control and comparison. This strain exhibits a high rate of mutation to the smooth or non-filamentous form. All attempts at isolation of lytic agents were carried out in triplicate using the James River strain and both the smooth and rough variants of ATCC 13338. The E. coli infected with B. bacteriovorus was isolated from a patient with urinary infection at Lynchburg General-Marshall Lodge Hospital.

Stock cultures of S. natans were maintained on Stokes medium (15) consisting of 0.2% glucose, 0.2% peptone and mineral salts. Transfers were made weekly alternating broth and agar media. Variations of this basic medium were made as noted.

Enrichment culture. Enrichment for lytic agents was carried out by adding 20 ml samples of water or sewage to a 24-hour culture of each of the test strains. This was further incubated for periods of 1 to 3 days. Following the removal of coarse particles, the supernatant was passed through a Falcon disposable filter of either 0.22 or 0.4 microns porosity (6). 10 ml of this filtrate was then added to a 24-hour culture of the appropriate strain, and 2 ml was added to the surfact of a Stokes agar plate as a check for sterility.

An alternate method (11) was used in the search for bacteriophage in which 3% v/v chloroform was added to the water or sewage samples before enrichment to decrease the bacterial count.

Since some phages require increased Mg ions for attachment, the $MgSO_4$ was increased to 0.002 M in these cultures.

Microculture. Microcultures were prepared by placing a drop of Stokes medium with 0.7% agar on a microscope slide. The bacteria were added to the top of the drop using the tip of an inoculating needle. A sterile cover slip was immediately placed over the drop, capillary air tubes placed in position to provide aeration and the edges sealed with Vaspar.

Microscopy. Cultures were examined with a Leitz Ortholux microscope with phase contrast equipment and recorded with a Leitz Orthomat camera using Kodak Tri X film. All magnifications are X2500 except where noted.

SECTION V

EXPERIMENTAL RESULTS

The filtrate from the enrichment cultures was added in 10 ml amounts to 24-hour cultures of the appropriate strain of Sphaerotilus and these cultures were observed at intervals for 2 weeks using phase microscopy.

After 2 days incubation a few very small motile forms could be seen in the fluid surrounding the filaments of Sphaerotilus. These do not photograph well because of their small size and active motility, but one can be clearly seen within the sheath of the James River strain in Fig. 1A. Some of the host cells can be seen in various stages of spheroplast formation. Fig.1B shows the same culture after 12 days when nearly all of the host cells show spheroplasting. The curved bodies inside the spheroplasts appear to be developing Bdellovibrio. The small circular inclusions may be storage granules of poly-beta-hydroxybutarate (15). The digestion of the contents of the host cell is considered to be a peicemeal process (13).

Passage of the filtrate through three successive host cultures results in an increase in both numbers and size of the bacterial parasite, as shown in Fig. 1C. This increase in size following the parasitic phase is one of the findings of Starr and Baigent (14). There was also a great increase of motility and avidity of attack. The initial attack appeared to be reversible with the parasite going from one host cell to another. After selection of the chosen host cell, the attack was extremely violent. On some occasions the parasite backed away several times before penetrating the host with a rotary movement which was relative to the point of attachment.

Fig. 1C also shows two spheroplasts with what appear to be emerging parasites, and one in which the parasite is clearly visible, but no cell contents can be seen.

Fig. 1D taken 4 days later shows many parasites attached to a filament of the host in which the cellular divisions are not sharply delineated. The reason for this is not clearly understood but may be due to the accumulation of exoenzymes which lyse the cell walls.

ATCC 13338 culture after 7 days following the addition of filtrate is shown in Fig. 2A. The cells of this strain are smaller than the James River strain and the spheroplasts appear relatively larger.

At the time the host cultures were inoculated with the filtrate, 2 ml were also added to the surface of a Stokes agar plate. The microcolonies shown in Fig. 2B appeared on the surface of this plate. Magnification is X300. It could not be demonstrated that these were colonies of host-independent Bdellovibrio. Some strains of the parasite can

be grown in cell-free media, usually requiring the addition of yeast extract (2 and 12), but the ability to change from one phase to the other does not appear to be a consistant property, and often can be demonstrated only with difficulty.

A 7-day old non-infected culture of the James River strain of Sphaerotilus is included for comparison in Fig. 2C.

Fig. 2D shows a culture of Escherichia coli infected with Bdellovibrio which was isolated from a patient with urinary infection, and is included here for comparison.

The second filtrate resulted in marked distortion of the cells of Sphaerotilus after 2 days incubation (Fig. 3A). After 6 days, nearly all of the cells are reduced to "ghost" forms which show faintly in Fig. 3B. Filtrate from these cultures produced a moderate growth on agar. The colonies were of moderate size, pearly in appearance at first, but gradually turning to bright yellow after several days. Microscopic examination revealed gram-negative rod-shaped bacteria often with bulbous ends and frequently appearing in clusters (Fig. 3C). They appeared to have a non-rigid cell wall. Round bodies of varying size frequently appeared among the clusters (Fig. 3D) These bacteria have been tentatively identified as Sporocytophaga myxococcoides. The bulbous ends are believed to be the beginning of microcyst formation which is represented by the round bodies. It will be noted in Fig. 3D that three of the round bodies closely resemble spheroplasts. Dworkin (5) reports the need for divalent cations for the formation of microcysts. The process is blocked by monovalent cations and results in the formation of spheroplasts.

The third isolate appeared as very tiny pinpoint colonies on the agar plate. After several transfers to the same media they seemed to adapt and formed small coral-red colonies. In microculture (Fig. 4A) they are seen as highly refractile beads and rods along a strand of Sphaerotilus. They are gram-negative. Fig. 4B shows the formation of oval microcysts. This organism has been tentatively identified as Sporocytophaga ellipsospora.

The fourth isolate formed very large extremely mucoid colonies on Stokes agar. It is a gram-negative rod. When the agar plate was incubated in an inverted position, the mucoid mass swarmed down the sides and into the cover. This isolate is also being further studied.

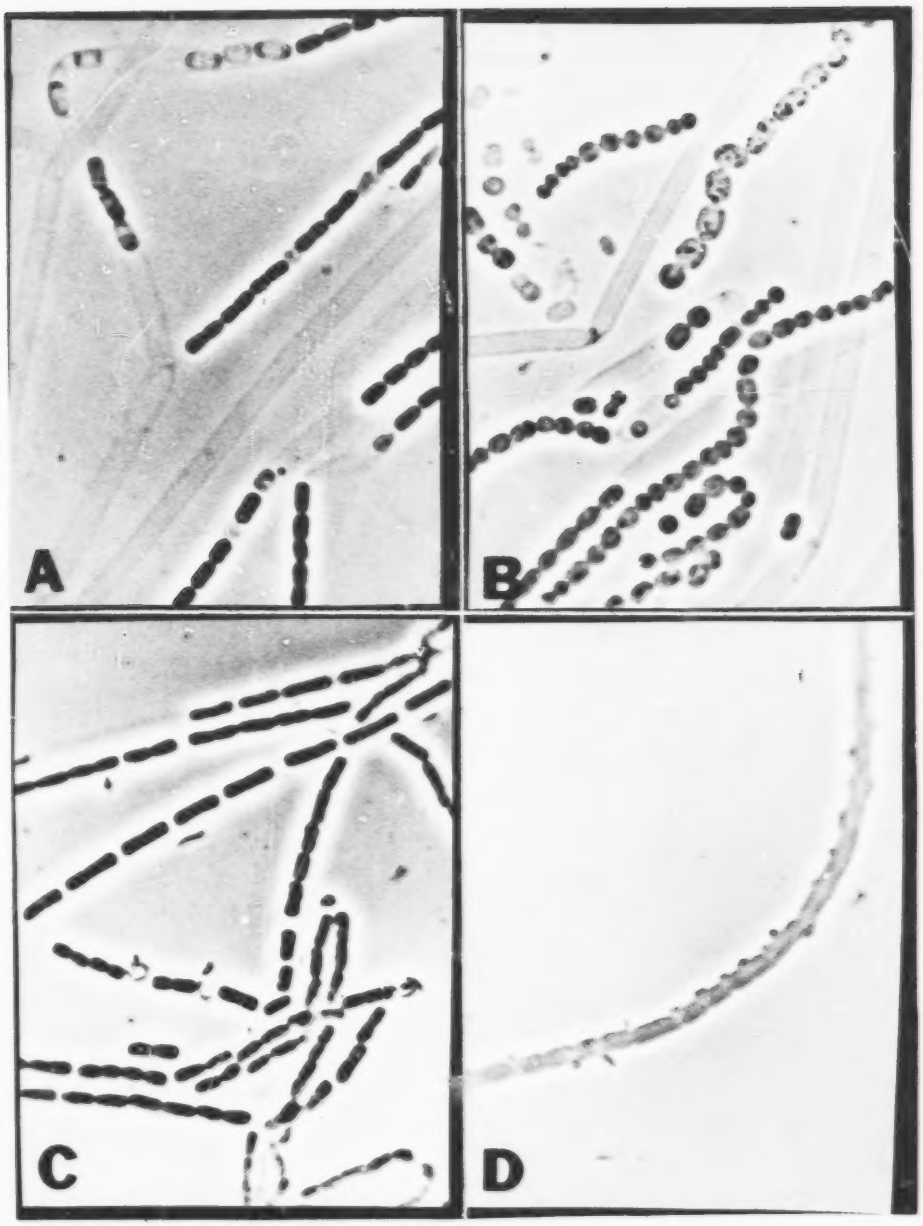

Fig. 1. James River strain of S. natans showing Bdellovibrio 2 days (A) and 12 days (B) after addition of enriched filtrate; 1 day (C) and 4 days (D) after 3 successive passages through the host.

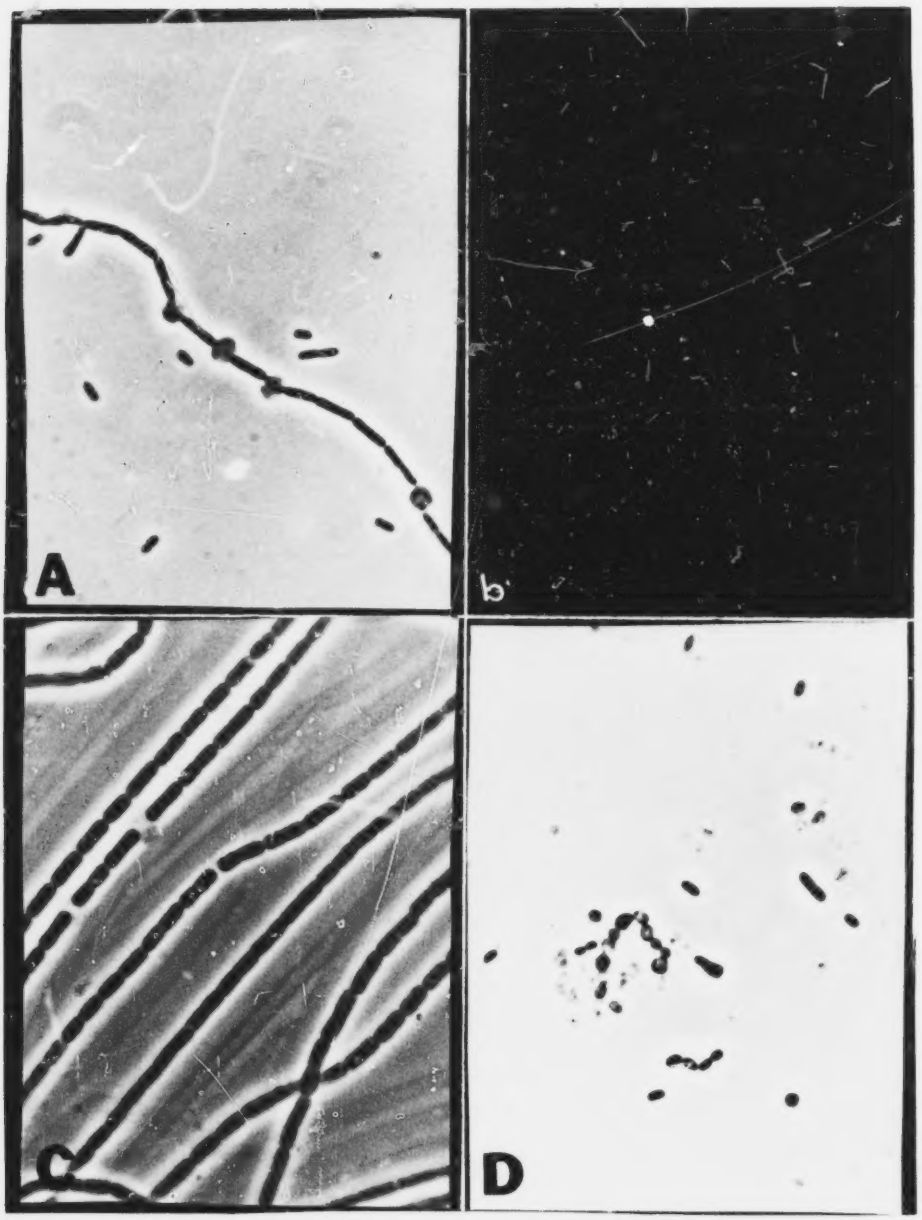

Fig. 1. ATCC 13338 strain of S. natans 7 days after addition of enriched filtrate (A); Microcolonies from agar plate (B); Uninfected culture of James River strain (C); E. coli infected with Bdellovibrio (D).

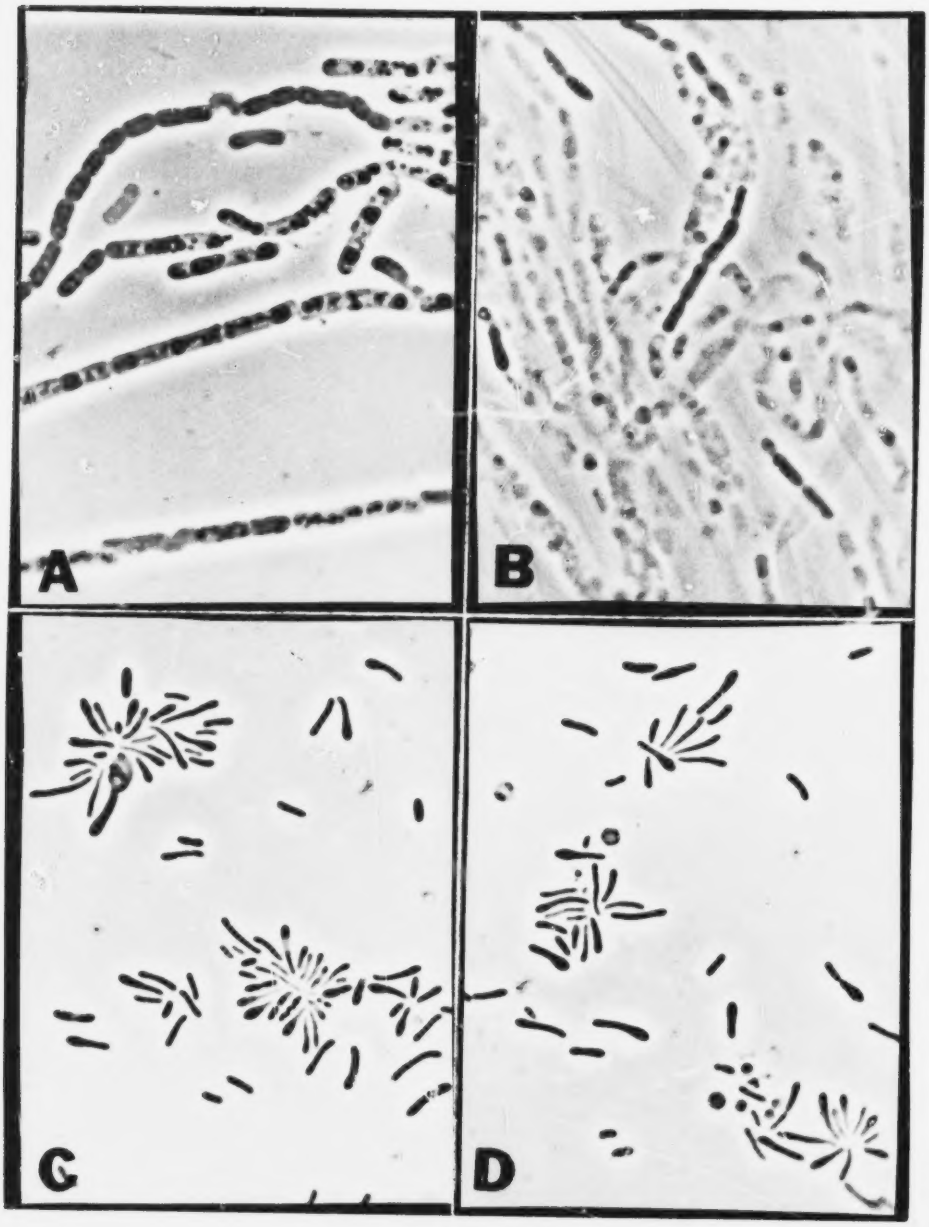

Fig. 3. Isolate tentatively identified as Sporocytophaga myxococcoides showing effect of filtrate on James River strain of S. natans after 2 days (A) and 6 days (B) incubation; showing clusters and bulb forms (C); showing microcysts and spheroplasts (D).

Fig. 4. Isolate tentatively identified as Sporocytophaga ellipsospora in microculture with filament of S. natans (A); showing formation of oval microcysts (B).

SECTION VI

DISCUSSION

The sequence of events recorded in Figs. 1 and 2 was consistant with parasitism by Bdellovibrio bacteriovorus. The initial enrichment showed very small motile forms and spheroplasting of the host cells. After 3 successive passages through the host, the parasites increased in size, motility, and avidity for the host.

These factors all concide with the descriptions in the literature, most notably that of Starr and Baigent (14) who state that B. bacteriovorus is an unusual bacterium which is predatory and parasitic upon other bacteria. The initial predatory phase involves the seeming "choice" of a susceptible host, followed by a violent contact between one or more of the highly motile Bdellovibrio cells. Shortly thereafter, all or part of the host cell becomes a spherical body, presumably a spheroplast. The ultimate results of this predatory and parasitic interaction are a complete disintegration of the host bacterium and an increase in the number of Bdellovibrio cells.

They also state that a most striking feature in the invasion of the host cell is the considerable increase in size of the individual Bdellovibrio cell during its parasitic stage. The fully nourished parasite may reach three or four times the volume of the original attacking parasite, particularly if the attacking parasite has been away from its host for some time. The work of Diedrich et al. (4) indicated that these larger forms which may be spiral in shape undergo subsequent fragmentation.

The filamentous nature of Sphaerotilus and its habit of growth in a mat at the surface of the broth medium do not allow a visual evaluation of the clearing of the medium. It appears that the cells may be lysed, but the sheath filaments remain intact. It is therefore only by microscopic examination that one becomes aware of the invader. Even in the relatively non-filamentous varient of ATCC 13338, an appreciable amount of turbidity remains. In neither case are all of the host cells killed, for Sphaerotilus can be subcultured from these seemingly dead cultures. These colonies do not appear to be resistant mutants, but rather bacteria which escaped parasitism. When the inoculum for subculture consisted of a colony on a piece of agar cut from the plate, the parasites were maintained in the new culture. When transfers were made by touching a loop to the filament ends in a young culture, it was possible to eliminate the parasites and return the culture to its uninfected state.

Another factor to be considered is the long generation time of Sphaerotilus in comparison with the enteric bacteria

and pseudomonads which have been the hosts for most of the Bdellovibrio that have been studied. This has been determined to be about 5 hours for the strain isolated from the Altamaha River in Georgia by A. H. Romano. (This data is included in Appendix B since it has not been published elsewhere.) It seems consistant that the parasitic phase takes place over a much longer period of time in Sphaerotilus than in E. coli.

The third consideration to be kept in mind is the fact that in isolating lytic agents from river water or sewage, there is a possibility that more than one such factor is present in a single enrichment culture. It is hoped that electron microscopy may be of help in this area by showing the intracellular parasite more clearly. The method for electron microscopy is included in Appendix A.

Other investigators have noted spheroplasting in Sphaerotilus (verbal communications) and suggested that this might be due to calcium deficiency. An experiment was carried out to test this possibility. Cultures were set up omitting the usual 0.005% $CaCl_2$ from the medium. It was calculated that they then contained 0.00013% Ca as chemical contaminants in the remaining ingredients in the medium. No effect on spheroplasting could be noted under these conditions. Dias and Dondero (3) conducted a study of the calcium requirements for growth and sheath formation of Sphaerotilus in continuous culture, but did not report the formation of spheroplasts. It should not be concluded from these studies that spheroplasting is never caused by calcium deficiency. It is obvious that anything that either blocked the formation of the cell wall or caused its destruction would result in spheroplasting and it is possilbe that several such factors may exist.

The isolation of the two species of bacteria tentatively identified as Sporocytophaga from the filtrate of enrichment cultures of Sphaerotilus and raw sewage is a logical result of their habit of growth on dung where they obtain their nutrition from other bacteria both living and dead. The ability to pass through a filter of 0.4 microns porosity is presumably due to the possession of a non-rigid cell wall. The formation of yellow and red non-diffusable pigment is consistent with this group of bacteria.

The very slow adaptation to growth on Stokes agar and the formation of both spheroplasts and microcysts can be explained by the very low nutritional content of this medium. Confusion exists in the literature concerning the differentiation of spheroplasts and microcysts. Dworkin (5) states that the latter must be capable of germination, resistant to physical disruption, refractile under the phase microscope and that the sequence of morphological

events leading to their formation must be clearly distinguishable from those characteristic of spheroplast formation.

These isolates represent an interesting and complex group of bacteria and must be studied further with continued application of Dworkin's findings on requirements for formation and outgrowth of microcysts before their relationship to Sphaerotilus can be evaluated.

SECTION VII

ACKNOWLEDGMENTS

The support of Randolph-Macon Woman's College is acknowledged with sincere thanks. Dr. William F. Quillian, Jr., President, Mr. V. Howard Belcher, Treasurer, and Dr. Franklin F. Flint, Chairman of the Biology Department, provided valuable assistance in obtaining laboratory space and equipment.

The constructive criticism of Dr. Antonio H. Romano, Department of Biological Sciences, University of Cincinnati, which was given during the course of this research is deeply appreciated.

The support of the project by the Federal Water Pollution Control Administration and the help provided by Mr. Cecil Chambers as the Grant Project Officer is acknowledged with sincere thanks.

SECTION VIII

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SECTION IX

PUBLICATIONS

Identification of *Sphaerotilus* Using Fluorescent Antibody Techniques

Abstract. The sheathed bacterium *Sphaerotilus natans* has been isolated from activated sludge a number of times, but evaluation of its importance in filamentous bulking of the sludge has been impeded by the difficulty in distinguishing it from other filamentous microorganisms such as *Bacillus cereus* var. *mycoides* and *Geotrichum candidum*. The sheath of *Sphaerotilus* consists of a protein-polysaccharide-lipid complex and was used to elicit antibody formation in the rabbit. The crude gamma globin was conjugated with fluorescein isothiocyanate. *Sphaerotilus* was identified in bicultures and in mixed populations from river water and from sewage by staining with the isothiocyanate-conjugated antibody.

Submitted to J. Bacteriol. for publication in the Notes section.

SECTION X

GLOSSARY

Bacteriophage - A virus that attacks and destroys bacteria.

Bdellovibrio - Gram-negative polarly flagellated bacteria which are predatory and parasitic on other bacteria.

Enrichment culture - Medium designed to selectively favor the growth of one kind of microorganism.

Fruiting bodies - Formations of myxobacters containing masses of microcysts.

Lysis - Disruption of cells.

Microcyst - Resistant, thick-walled resting forms of myxobacteria.

Mutant - An organism with a changed or new gene.

Myxobacteria - Gram-negative rods with non-rigid cell wall, motile by gliding, produce microcysts which are often borne in fruiting bodies.

Spheroplast - Bacterial cells which have lost cell-wall substance.

APPENDIX A

Electron Microscopy of *Bdellovibrio bacteriovorus*

Adapted from: Burnham, J. C., T. Hashimoto, and S. F. Conti. Electron microscopic observations on the penetration of *Bdellovibrio bacteriovorus* into gram-negative hosts. J. Bacteriol. 96:1366-1381.

Kellenberger, E., A. Ryter, and J. Sechaud. Electron microscope study of DNA-containing plasms. J. Biophys. Biochem. Cytol.

Michaelis veronal-acetate buffer:

veronal	2.94 g
sodium acetate	1.94 g
sodium chloride	3.4 g
dist. water to	100 ml

Kellenberger buffer:

veronal acetate buffer	5.0 ml
dist. water	13.0 ml
0.1 N HCl	7.0 ml
1M Ca Cl_2	.25 ml

The pH is adjusted to pH 6.0 with the HCl. Make up fresh on the day of use.

1. Samples mixed with glutaraldehyde (buffered at pH 6.1 with modified veronal acetate buffer) to a final aldehyde conc. of 1%.
2. Cells centrifuged and suspended in 6.25% glutaraldehyde in modified veronal acetate buffer, for 12 to 24 hours.
3. Wash 3 times in modified veronal acetate buffer.
4. Fix for 12 hours in 2% osmiun tetroxide in modified veronal acetate buffer at pH 6.1.
5. Samples suspended in 0.5% aqueous uranyl acetate.
6. Dehydrate in graded acetone series.
7. Infiltrate with a mixture of Epon 812 and 815.
8. Polymerize at 60° for 24 hours.
9. Section and place on 300 or 400 mesh copper grid.
10. Postain with uranyl acetate and Renold's lead acetate.

APPENDIX B

Sphaerotilus natans Growth Curve

From: Geason D. J. Sheath formation in Sphaerotilus natans followed by immunofluoresence. Masters dissertation, University of Cincinnati Graduate School.

Growth curve studies were done to relate sheath synthesis with growth, and, in subsequent experiments, to relate incubation times with generation time.

The filamentous nature of S. natans necessitated a modification of the usual methods of following growth. The tendency of the filaments to adhere to each other preclude removal of aliquots of the culture for counting, and plate counts would be valueless because colonies do rise from single cells. Estimates of generation time based on total weight would be inaccurate because of the variation in cell-sheath ratio.

Stokes (1954) reported optimum growth at "about" 30°C, and Hoflick (1901) reported 25 to 35°C as the optimum range, but both of these estimates were based on visual observations. Preliminary experiments on labeling of the sheath had shown good results with 28°C as the incubation temperature. Therefore, this temperature was selected for growth studies and does not necessarily represent optimum growth temperature.

Two methods based on removal of the sheath with hot TCA were devised to follow cell number. The first method was a direct count of the organisms, and the second method entailed measuring the increase in turbidity.

Direct count. A 72-hour culture of S. natans in Stokes media was agitated in a Waring blender for two minutes. Of this suspension, 0.4 ml was used as an inoculum for 100 ml of Stokes medium. After thorough mixing, 5 ml portions were despensed into sterile tubes and incubated at 28° C for periods of time ranging from 6 to 48 hours. At the desired time intervals, duplicate tubes were removed from the water bath. At this time, 5 ml of 10% TCA was added to each tube, and they were heated to 90°C for 10 minutes. This treatment dissolved the sheath and freed all of the cells, including those which had previously adhered to the side of the tube. After the cells had been sedimented by centrifugation at 8,000 rpm for 15 minutes, they were resuspended in 5 ml portions of saline. After thorough mixing,0.01 ml was removed and placed in an area of 1 square cm on a slide in the usual manner for a direct count. After staining with methylene blue, the cells were counted with a calibrated microscope, and the results plotted against time, as shown in Fig. I.

Turbidity method. A growth curve based on turbidity was determined in a similar manner except that after re-

suspending the cells in saline, turbidity was measured by per cent light transmission in a Beckman spectrophotometer. The results were converted to optical density and were plotted against time. The resulting graph is shown in Fig. II.

The difference in the curves obtained by the two methods was most evident during the early log phase and was considered due to the fact that the cells were dividing rapidly, and therefore were small in size.

The direct count method was considered to be more relevant to the subsequent experiments with fluorescent antibody labeling of the sheath since the object was to correlate sheath formation with cell division rather than cell size.

Generation time by the direct count method was found to be 5.5 hours for the time period from 5 to 30 hours. This time period was chosen because it included the time range to be used in the labeling experiments.

The data collected in both experiments is presented in Table I.

TABLE I

GROWTH CURVE DATA

Hours Incubation	Optical Density Average 2 tubes	Direct Count Average 10 fields
0	.01	3.3×10^5
5	.03	4.0×10^5
12	.013	2.3×10^6
23	.043	5.8×10^6
30	.060	9.6×10^6
36	.104	1.8×10^7
48	.170	3.5×10^7

FIGURE I

GROWTH CURVE: DIRECT COUNT

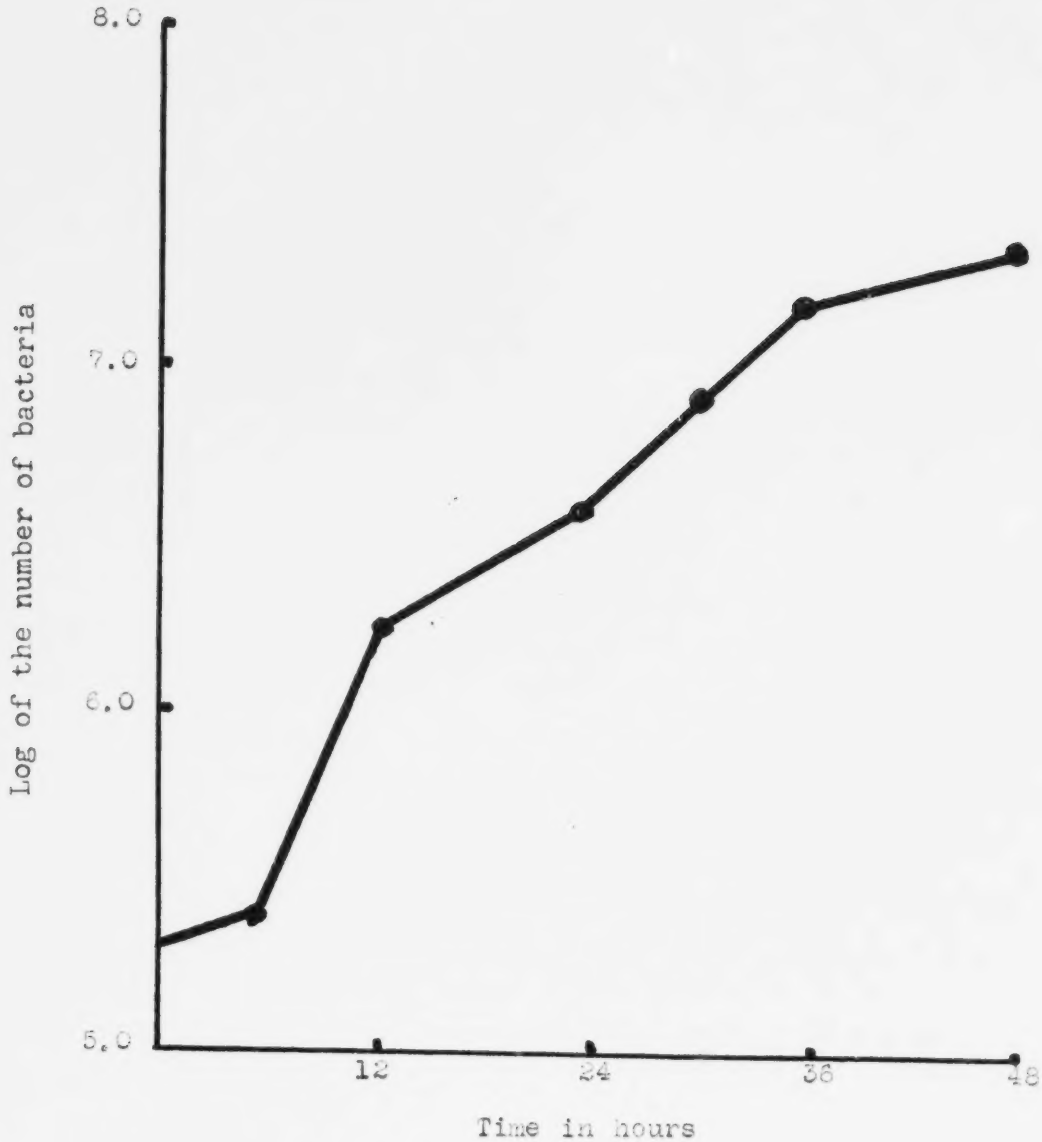
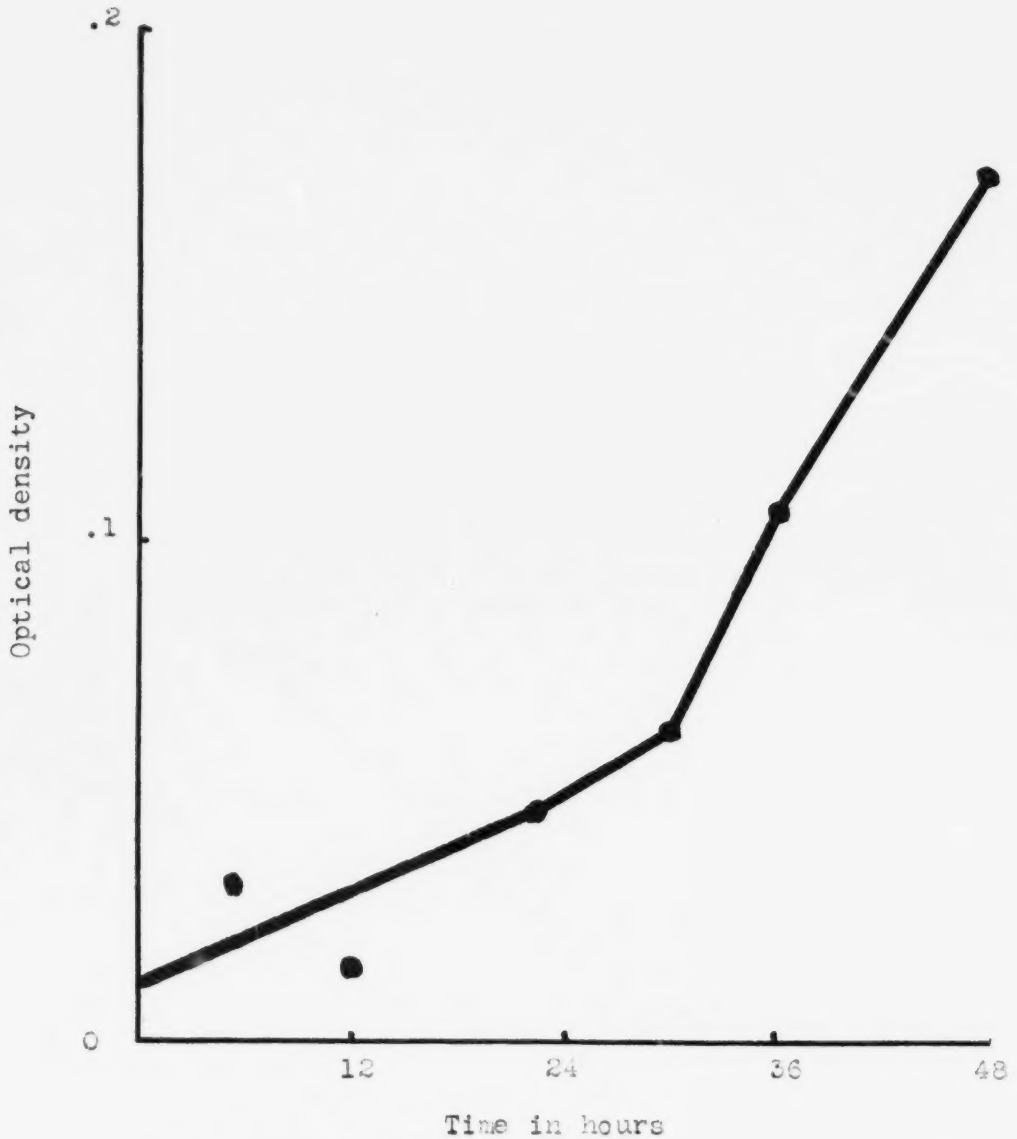


FIGURE II

GROWTH CURVE: TURBIDITY



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ACCESSION NO.

ABSTRACT

The filtrate from enrichment cultures of raw sewage or river water and *Sphaerotilus* was used to isolate *Bdellovibrio* causing spheroplasting and lysis of the host cells but not the sheath. The size and avidity of the parasite increased. Two myxobacters and an unidentified gram-negative rod also caused lysis of *Sphaerotilus*. Specific bacteriophage could not be demonstrated.

KEY WORDS:

Sphaerotilus
Bdellovibrio
spheroplast
lysis
myxobacters
bacteriophage